# Is immunoglobulin A anti-tissue transglutaminase antibody a reliable serological marker of coeliac disease?

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***Background*** **Anti-tissue transglutaminase (tTG) antibody is being used increasingly as a diagnostic tool in the serological investigation of coeliac disease. However, positive predictive values of immunoglobulin A (IgA) anti-tTG for coeliac disease in prospective studies have been disappointing and false-positive results are reported.**

***Objective*** **To assess the clinical utility of cascade testing for anti-tTG and anti-endomysium antibody (AEA).**

***Patients*** **Two unselected retrospective cohorts from routine diagnostic investigation for possible gluten sensitive enteropathy: group 1 comprised 57 cases seropositive for anti-tTG and group 2 comprised 52 cases seronegative for anti-tTG. In both groups, all cases had also undergone small-intestinal biopsy.**

***Methods*** **Patients were assessed for the presence of IgA anti-tTG by enzyme-linked immunosorbent assay and for IgA AEA by immunofluorescence.**

***Results*** **The positive predictive value of IgA anti-tTG for biopsy-confirmed coeliac disease was 54%. The positive predictive value of dual positivity for anti-tTG and AEA was 97%. The negative predictive value of IgA anti-tTG was 100%.**

***Conclusions*** **The data presented here support the use of IgA anti-tTG as an initial screen for coeliac disease. Coeliac disease is unlikely when IgA anti-tTG is absent. However, many false-positive results are seen, and clinical utility and diagnostic efficiency are improved markedly if positive results are confirmed with the more accurate, but labour-intensive, AEA assay.** ***Eur J Gastroenterol Hepatol*** **16:467–470 © 2004 Lippincott Williams & Wilkins**

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## Introduction

Serological investigations are helpful in identifying cases of coeliac disease. However, small-intestinal biopsy remains the gold standard investigation and should be performed in cases of high clinical suspicion, even when the serology is negative.

Tissue transglutaminase (tTG) has been shown to be a major antigen in the autoimmune response seen in coeliac disease [1]. Since this initial report, it has been shown that anti-tTG, anti-R1 reticulin, anti-jejunum and anti-endomysium (AEA) are very closely related (if not identical) specificities [2,3]. As a result, various enzyme-linked immunosorbent assays (ELISAs) have been developed [4–8], showing immunoglobulin A (IgA) anti-tTG to be a useful tool in the serological investigation of coeliac disease, with both high sensitivity and specificity. However, positive predictive values (PPVs) of IgA anti-tTG for coeliac disease in prospective studies have been disappointing (22–67%) [9,10], bringing the clinical utility of the assay into question. In contrast, the PPV of IgA AEA has been consistently high (91–100%) [11].

Suspecting that false-positive anti-tTG results were commonplace, we evaluated our own practice. We present data on the positive and negative predictive values of IgA anti-tTG alone and as part of a cascade with IgA AEA.

## Methods

### Study groups

We examined retrospectively the data on sera submitted routinely to the immunology laboratories at Southmead Hospital, Bristol, UK, and the Gloucester Royal Hospital, Gloucester, UK, during 6 months of 2001. Computerised hospital records were interrogated post-serological testing to ascertain small-bowel biopsy results. Patients who were seropositive for IgA anti-tTG and proceeded to small-bowel biopsy constituted

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group 1. Similarly, patients during the same period who were seronegative for anti-tTG but who nonetheless had proceeded to small-bowel biopsy constituted group 2.

#### Routine screening for coeliac disease serology

Samples submitted for coeliac disease investigations were screened for the presence of IgA class anti-human tTG antibodies by means of a commercial ELISA (Orgentec Diagnostika GmbH, Mainz, Germany). All sera found positive on this initial screen were assessed by immunofluorescence for the presence of IgA AEA using monkey oesophagus (Binding Site, Birmingham, UK), as described previously [11].

In order to confirm the quality of the Orgentec assay, some of the sera were also analysed by two other commercial ELISAs (90 sera by Aeskulisa anti-human-tTG kit (Aesku.Diagnostics GmbH, Wendelsheim, Germany) and 58 sera by Bindazyme IgA anti-guinea pig-tTG kit (Binding Site)) according to the manufacturers' instructions.

#### Histological assessment

Small-bowel biopsies were considered to be consistent with a diagnosis of coeliac disease if total or marked but partial villous atrophy and crypt hyperplasia were present in association with increased numbers of intra-epithelial lymphocytes. Consultants in histopathology routinely interpreted biopsies on both sites.

This study was undertaken as a retrospective audit of current practice. Our local ethics committee confirmed that formal ethical approval was not required. All patients gave consent for the serological and, where performed, small-bowel investigations.

Statistical analysis was by linear regression or chi-squared analysis, as appropriate.

## Results

A total of 1655 routine serum samples were tested over the 6-month period. Of these, 176 (10.6%) were positive for IgA anti-tTG. Within this group, 47 (26.7%) were also positive for IgA AEA. Of the 176 patients who were seropositive for IgA anti-tTG and therefore considered suitable for analysis, irrespective of AEA status, 57 consecutive patients were found who had proceeded to small-bowel biopsy (group 1) (Table 1). The PPV is given by the ratio of seropositive patients with coeliac disease to all seropositive patients, expressed as a percentage. The PPV of IgA anti-tTG for biopsy-confirmed coeliac disease in group 1 was only 54% (31/57). The PPV of dual positivity for IgA anti-tTG and IgA AEA for biopsy-confirmed coeliac disease was 97% (28/29). Group 2 comprised 52 patients who were seronegative for anti-tTG but who had nonetheless proceeded to small-bowel biopsy. Eighteen of these 52 patients showed slightly abnormal biopsy findings, but in none of these 18 cases was the histology compatible with coeliac disease. The negative predictive value is given by the ratio of seronegative patients without coeliac disease to all seronegative patients, expressed as a percentage. Thus, in the 52 cases, the negative predictive value of anti-tTG for coeliac disease was 100% (52/52) (Table 1).

Correlation between the Orgentec kit (human tTG) and the Binding Site kit (guinea pig tTG) was 0.92 ($P < 0.001$). Correspondence (i.e. both kits giving the same qualitative result, either positive or negative) between the Orgentec kit and the Binding Site kit was 91%. Of the five discordant samples, four were positive using the guinea pig substrate and negative using human substrate. Correlation between the Orgentec kit and the Aeskulisa kit (human tTG) was 0.92 ($P < 0.001$). Correspondence between the Orgentec kit and the Aeskulisa kit was 97%.

## Discussion

Recent data from UK National External Quality Assessment Schemes (NEQAS) indicate that 109 of 253 (43%) laboratories registered use IgA anti-tTG in their investigations for possible coeliac disease. The three most popular commercial assays are Inova (22/109), Orgentec (17/109) and Pharmacia ELISA (17/109). In

**Table 1 Results of small-bowel biopsy data, anti-tissue transglutaminase (tTG) antibodies and anti-endomysium antibodies (AEA)**

| | | Biopsy data | | IgA AEA | |
|---|---|---|---|---|---|
| Anti-tTG | Patients (*n*) | Diagnosis | Patients (*n*) | Negative | Positive |
| Positive (group 1) | 57 | CD | 31 | 3 | 28 |
| | | Abnormal, but not CD | 7* | 7 | 0 |
| | | Normal | 19 | 18 | 1 |
| Negative (group 2) | 52 | CD | 0 | NT | NT |
| | | Abnormal, but not CD | 18 | NT | NT |
| | | Normal | 34 | NT | NT |

*The majority had non-specific duodenitis, one case had giardiasis, and one case had Crohn's disease.
CD, coeliac disease; IgA, immunoglobulin A; NT, not tested.

this study, we evaluated the clinical utility of the Orgentec IgA anti-tTG in routine screening for coeliac disease. No disease sensitivity, specificity or PPV data are available in the literature supplied with the kit.

Initial concerns were that our routine assay (Orgentec) was not specific for anti-tTG, since only 26% of positive assays were confirmed with AEA. In some assays, antigen purity may be a problem. However, this kit uses human recombinant tTG, and therefore this should not be an issue. Comparison with two other commercial assays (Binding Site and Aeskulisa) showed similar quantified results ($r = 0.92$ and $r = 0.92$, respectively) and qualitative correspondence (91% and 97%, respectively). Correspondence was noted to be higher between the two kits based on human antigen, suggesting that even more false-positive results might be expected with guinea pig substrate. In addition, UK NEQAS data suggest that all anti-tTG ELISA used give broadly the same results.

The high false-positive rate may, in part, be the result of the commercial assay cut-off being incorrect. This has been described previously for a number of kits [12]. It is also possible that novel (but irrelevant) epitopes are expressed when the antigen is purified and fixed artificially on an ELISA plate, as compared with the native antigen *in situ* (tissue section of monkey oesophagus). This has been seen previously with antibodies to extractable nuclear antigens, where 'natural' conformational antigens may be expressed in some systems but not others [13]. Further, recent studies report that positive anti-tTG may be a relatively common (non-specific) finding in cirrhotic patients [14] and in patients with end-stage heart failure [15] where there is no clear evidence of coeliac disease.

We confirm that the PPV of IgA anti-tTG in routine screening is far from ideal. Data from group 1 (Table 1) show a 54% PPV (31/57). Many (119/176) of our IgA anti-tTG positive cases surprisingly did not proceed to biopsy. It is possible that in those cases not biopsied, the index of clinical suspicion was very low and thus many of these may be false-positives. Hence, the worst-case scenario would be 18% PPV (31/176; all confirmed cases of coeliac disease/total anti-tTG positives in study). Our data (PPV 18–54%) encompass those published previously, e.g. Chan and colleagues [10] (PPV 67%) and Bardella and colleagues [9] (PPV 22%). PPV, unlike sensitivity and specificity, is highly dependent on ascertainment criteria. Early figures, such as our own 92% [5], were based on highly selected and well-documented patient groups with a known high prevalence of coeliac disease. Our more recent studies reflect the use of anti-tTG in routine practice, where the prevalence of disease is lower. Interestingly, we found the PPV of AEA in our present study to be maintained (97%), as compared with our previous study (96–100%) [5]. The high negative predictive value of anti-tTG reported here suggests that few true coeliac patients will be missed if anti-tTG is used alone as a primary screen (one exception being IgA-deficient coeliac disease patients). Note also that some studies reported a significant percentage of coeliac disease patients who are negative for anti-tTG at presentation [16]. We, however, have seen very few patients with this serological picture, and none emerged during this study.

The present report, indicating diminished PPV of IgA anti-tTG for coeliac disease when using the IgA anti-tTG assay widely in a district setting, is not surprising. The same phenomenon is recognised, for example, with anti-neutrophil cytoplasm antibody (ANCA) testing [17,18], despite initial reports of high specificity and sensitivity published by tertiary referral centres [19,20]. Findings are again compatible with the reduced disease prevalence in the general, as compared to a highly selected referral, population.

The sensitivity of IgA AEA based on the data from group 1 (28/31, 90%) is slightly lower than in our original study. Three sera from patients with coeliac disease were positive for IgA anti-tTG but negative for IgA AEA at diagnosis. This phenomenon has been described previously [16].

The data presented here support the use of IgA anti-tTG as an effective screen for coeliac disease. However, clinical utility is improved if positive results are confirmed with the more accurate, but labour-intensive, AEA assay.

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**Conflict of interest**

None declared.

**Authors' contribution**

D.U. conceived the project. R.L. and S.S. performed laboratory analyses and computer searches. All authors were involved in data analysis and write-up.

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